TIMELINE

JUNE

2nd:

- Met team members (Yay!)
- Brainstormed Team logo
- Each team member was given an iGEM brooch to cherish.
- Watched a sad documentary on Malaria and got inspiration to start off our project

https://www.youtube.com/watch?v=HL26nz31hEQ

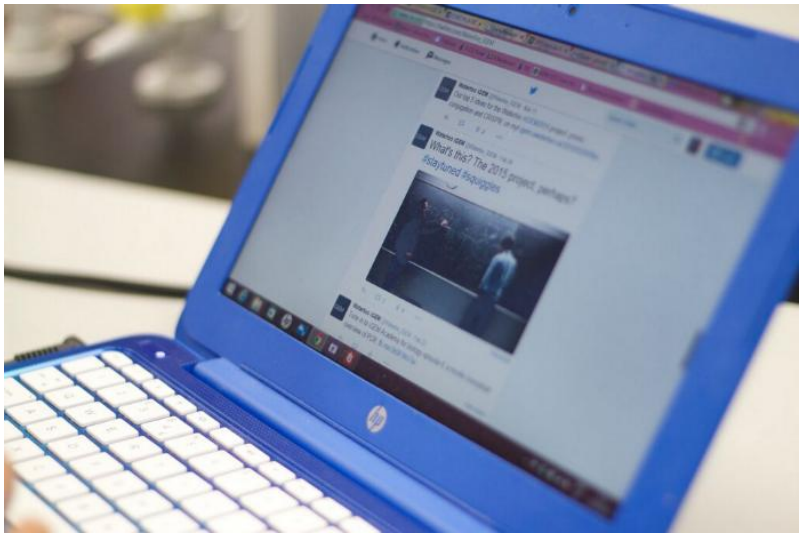
3rd:

- Started lab work in the Genetics lab with instructors Matt and Killian.
- Plasmids with RFP biobricks assembled as practise for the main protocols that would be carried out later during the summer.
- Also learned how to make LB agar, carry out gel electrophoresis and agar plates.

5th:

- Team members showed off their presentation skills by giving short talks on previous winning iGEM teams.

- The idea for our YouTube channel iGEM Academy was born!
- Started work on Human practises by researching literature on Malaria and gender inequality.



6th:

- More research work on Artemisinin resistance and semisynthetic artemisinin.

8th:

- During practise lab sessions, the group was divided into boys and girls teams.

10th:

- Came up with the final draft of team logo.

11th:

- Competition run by one of our instructors (Killian) to test our procedure precision. Everybody was a winner in the end!

12th:

- Went to the library and had a tutorial with Ben to operate Geneious program.
- Sent email to Camden Clothing to ask about printing of iGEM logo on t-shirts.
- Also sent email to Wiser@tcd to get statistics of College academics gender distribution for our analysis on Gender inequality in Science and Technology.

13th:

- Wrote out drafts for scripts and storyboards for our iGEM Academy video channel.

15th:

- Team moved to BioLab 3! Hurray!

17th

- Went to Camden clothing with the whole crew to get order for our iGEM shirts.
- Made our first batch of competent cells for Interlab study using the Genetics lab protocol.

19th:

- NO colonies observed after transformation of competent cells. Our first failure. Sad day!
- Brainstormed solutions.
- Finished shooting protocols' video 1,2,3,4 and 10 for iGEM academy.
- Edited videos using iMovie.

22nd:

- Carried out first transformation efficiency protocol to check if our competent cells were competent.

23rd:

- No colonies observed showing our competent cells were incompetent.
- Started making new competent XLI-BLUE cells using iGEM competent cells protocol.

26th:

- Photo day with the group with our new shirts!

27th:

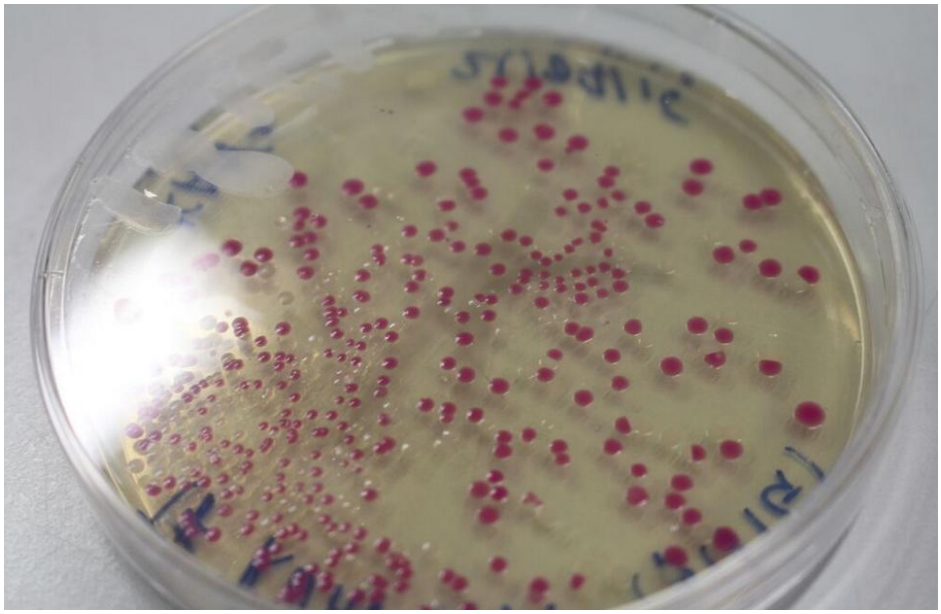
- Recorded audios for iGEM academy channel videos.

29th:

- Started using Twitter page on a regular basis. #iGEM2015

30th:

- Competent cells worked! Red colonies observed of RFP!



- Started research on Global Health issues and Malaria for Human Practises. Decided the layout of the human practises tab.

JULY:

1th:

- Agreed to be in the newsletter with Amoy iGEM. Wrote an article about iGEM academy as collaboration platform in News Letter 7.

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Team - TrinityCollegeDublin

- Project Introductions: a platform for teams to share their project ideas, work done so far and goals for the future.
- iGEM Tutorials: videos that provide visitors with up to date tutorials on synthetic biology and lab techniques that are relevant to synthetic biology. Our set of lab tutorials 'A Beginner's Guide' has already been uploaded, make sure to check it out!
- Lab Art: a showcase of the colourful and creative world of synthetic biology
- Musical iGEM: a celebration of song and dance in the lab.
- Outside the Lab: videos outlining synthetic biology work being done outside of the competition
- iGEM HQ: videos about the iGEM competition and the Giant Jamboree.
- Let's Talk: video interviews with members of the public talking about synthetic biology

Taking part is easy and straightforward. Some iGEM teams with pre-existing Youtube channels have given permission for their videos to be linked to from the iGEM Academy channel.

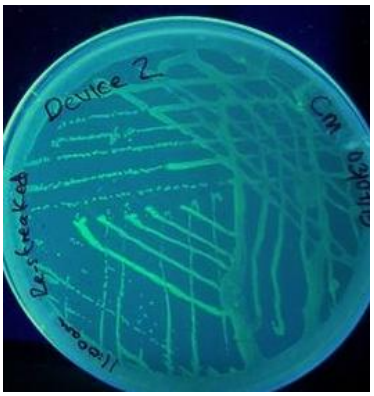
Other teams have become managers of the channel allowing them to upload videos directly to the iGEM Academy channel.

Currently, the aim for the future is to grow iGEM Academy so that it will become a source of information for future iGEM teams as well as other visitors looking to find out more about the iGEM competition and synthetic biology. It is important to point out that this is a joint effort. We hope that as many teams as possible take part in helping to make iGEM Academy a base which connects viewers with the YouTube channels and videos of all iGEM teams.

Contact igemacademy@gmail.com for more information.

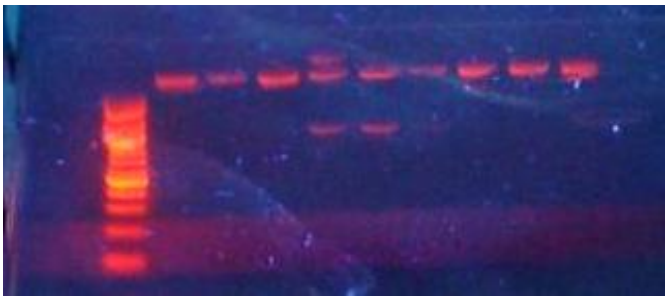
3rd:

- Got numerous colonies for the Interlab study devices. Success!
- Got a chance to view all the protocol videos we did for the iGEM Academy channel in the conference room. Had celebratory cake (Yummy!).



7th:

- Carried out our first Gel electrophoresis for the Interlab study constructs. It wasn't our best gel but it worked!



9th:

- Apart from the Interlab Study in progress, we had a collection of various lab artwork by now.

10th:

- Had Pizza and doughnuts with Faculty of Engineering, Maths and Science. Told them about our project.
- More audio recorded for our YouTube channel, iGEM Academy.

14th:

- The iGEM Academy slogan and script for the introductory video was decided upon.
- Our broth cultures for the Interlab Study were ready to be inoculated.

15th:

- Our DNA arrived thanks to IDT DNA!

- Recorded all of the team members' voice overs for the intro video of iGEM Academy.

20th:

- Uploaded all the protocol videos to iGEM Academy.
- Went to the Flow Cytometry Facility for our final Interlab Study measurements.

21st:

- 100+ followers on Twitter!
- Our lab equipment arrived. It was a happy day!

22nd:

- Official Meet up with Cork iGEM in Trinity College Global Room. Gave Cork Team members a tour of the campus and discussed possible collaborations.

26th:

- Email was sent to all iGEM teams' Twitter accounts asking for collaboration for iGEM Academy.

AUGUST

3rd:

- Received the primers we ordered from Sigma Aldrich.

4th:

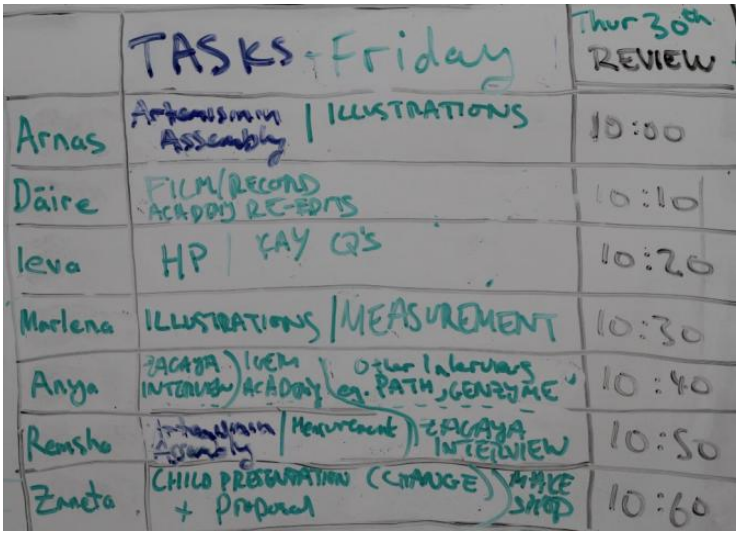
- All of our iGEM Academy protocol videos (after editing) got uploaded to YouTube.
- We carried out the first PCR of our parts: ALDH1, DBR2 and ADH1.

5th:

- Finalised how our web pages for the wiki are going to look like. We decided on having triangles as our building blocks because triangles are awesome!
- Our B569 cells arrived from Amyris. Exciting stuff!

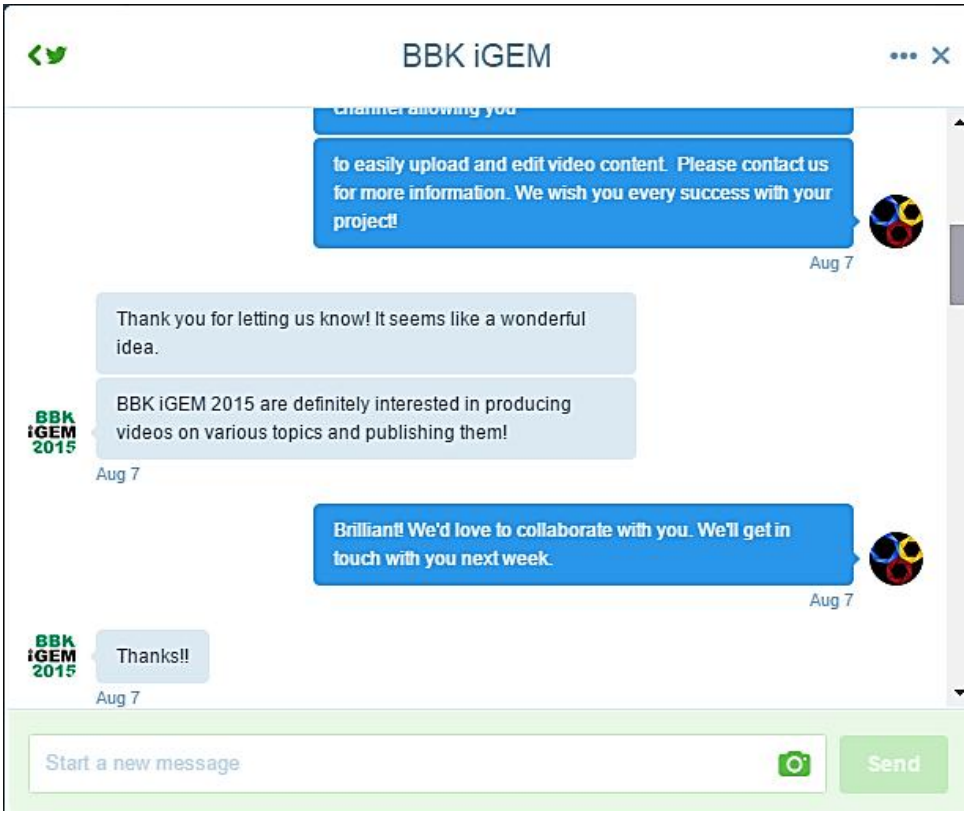
6th:

- Held team meeting and discussed medal requirements and designation of tasks.



7th:

- Birkbeck iGEM was our first collaborator for iGEM Academy

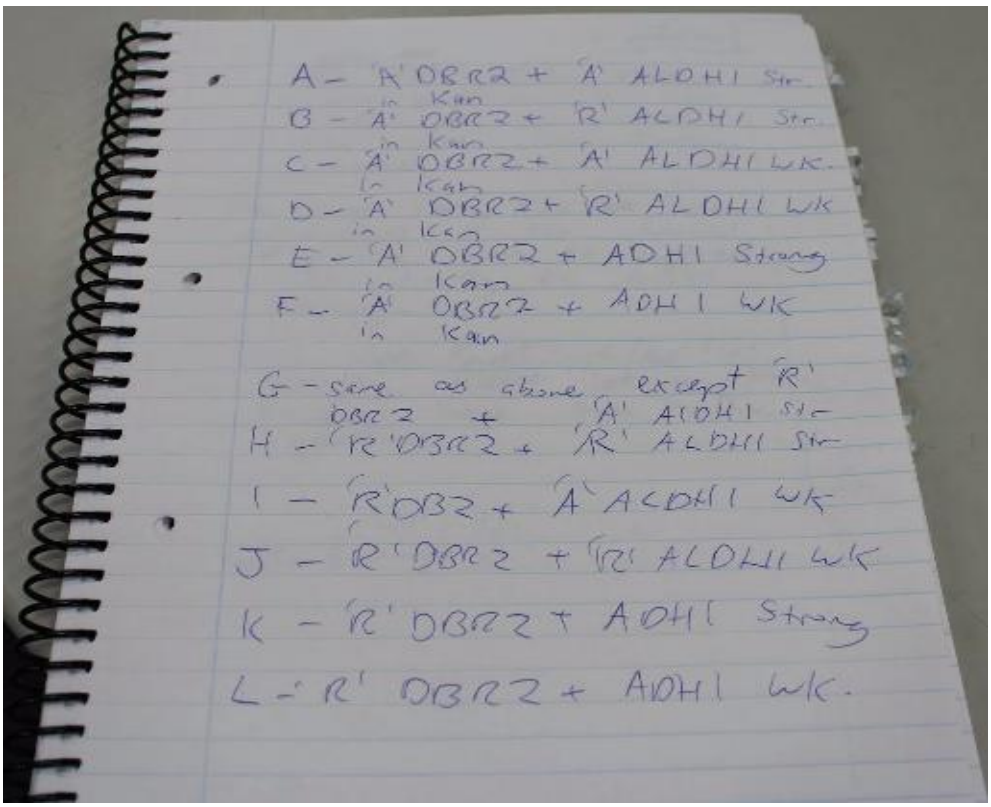


10th:

- Our Skype Interview with Kathleen Monroe of Zagaya took place. It was a valuable interview for our human practises work as she talked about her experience of Zagaya, semisynthetic artemisinin and distribution.

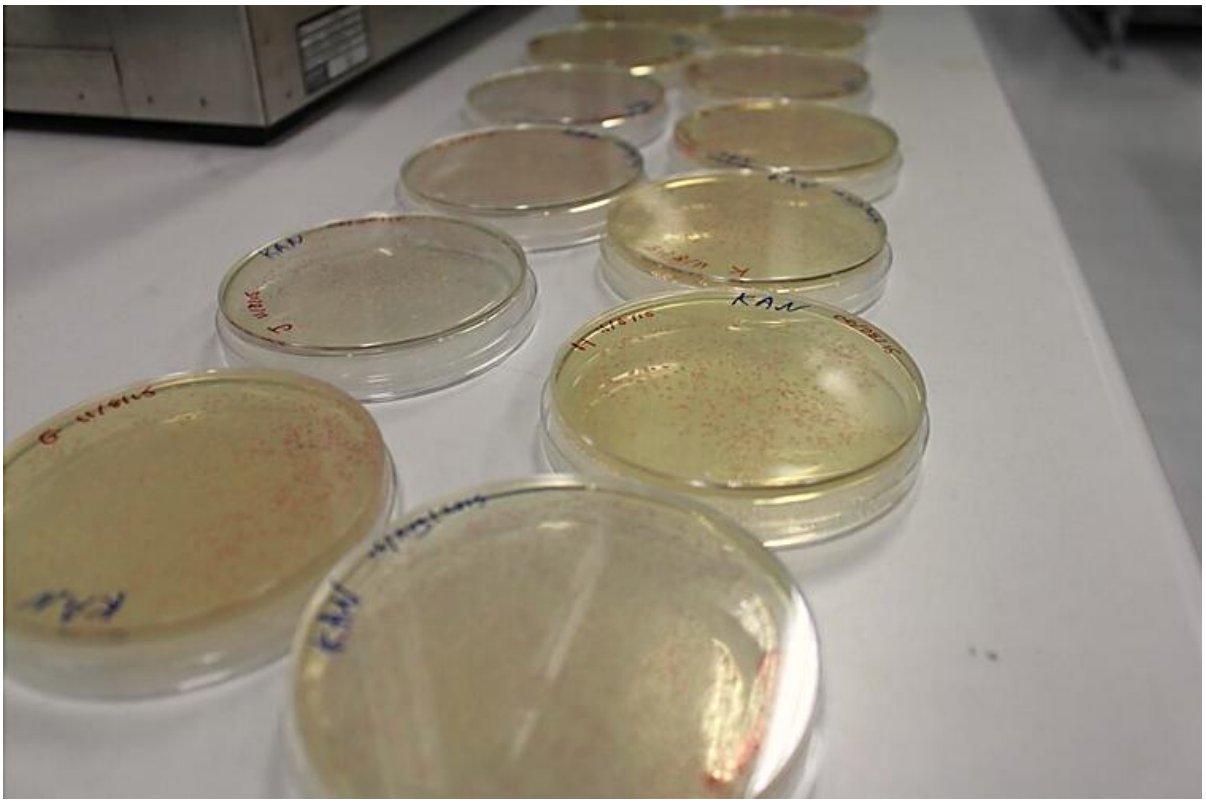
11th:

- Carried out restriction, ligation and transformation to add genes with RBS and promoter/terminator together to get minimal constructs. (Promoter + RBS + DBR2 + ALDH1 + RBS + Term). We developed a coding system for our different combination of constructs:



12th:

- Other teams such as iGEM Valencia, Oxford, Groningen, Ionis and more agreed to be part of iGEM Academy.
- Had interview with Zagaya's Chris Paddon
- All plates developed colonies from the previous day's transformations!



13th:

- Unfortunately, due to some mix up with one of the promoter tubes we found out that we used the wrong promoter (taught us a lesson in labelling properly!). Also, due to the scar created by BioBrick assembly there was no feasible way for us to insert a new promoter between the GFP gene and DBR2. The sequencing results that had been carried out after the addition of RBS found that some samples (R2) that had been used to add further parts, had no DBR2 gene also.

- We decided to run a gel the following day and start over after finding out the correct identity of the promoter.

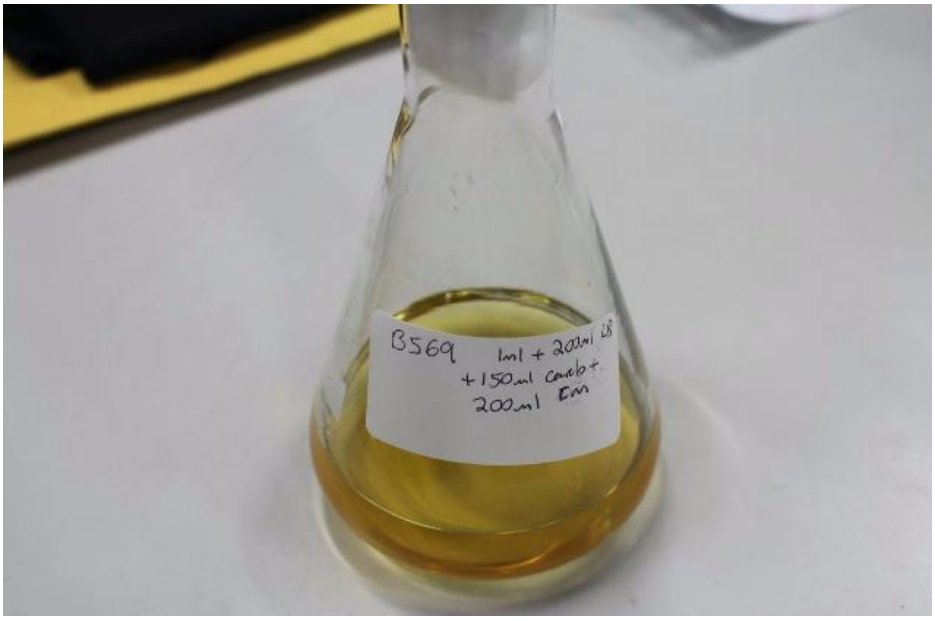
14th:

- The gel we ran showed us the right promoter D2 that we needed to use, and so we carried out the 3A assembly protocol all over again over the next 2 days.

- We also brainstormed questions to ask Ingrid Chen from Malaria Elimination Initiative, UCSF for our human practises work over some Pizza and the film GATTACA.

18th:

- Skype call with Ingrid Chen.
- Preparation of B569 Broth



19th:

- Preparation of competent cells B569 cells.

- Decided to repeat the digestion and ligation carried out on 17/8/15 due to overall poor growth. We had little miniprepped RFP Kan left so we decided to use the linearized RFP Kan (pSB1K3) from the distribution kit instead. We decided to carry out the whole process again and then to also do a duplicate mixing new digestions with ones from the 17th that had been stored on ice and then a triplicate with entirely old ligation mixes.

20th:

- B569 transformation carried out. Small, white colonies on all plates.

- We carried out a miniprep on the RFP broths, D2 promoter broth and min weak and strong broths that had been prepared the previous day.

- We tested the concentration of DNA in the minipreps and found no or very little DNA in what we had thought to be the min weak and strong minipreps. As the plates they had originated from had been left in the incubator for a day longer than usual we hypothesised that the antibiotic had broken day and that what we had hoped were white colonies containing our construct were really unknown infections on the plate.

21st:

- Synthetic Biology Tutorial for kids!

24th:

- Carried out the start of the transformation of the PTAC white and red (k864400) (there was unexpectedly white and red colonies on the plate that had been transformed directly from the well in the kit plate and we wanted to try and clarify the identity of them by putting them on a selective plate) minipreps and the miniprepped Max Weak new.

- Carried out the freeze-thaw protocol for the B569 constructs that had been removed from the fermenter.

25th:

- Preparation for GC/MS.
- Discussed how our team banner will look like.

26th:

- Digestion and Ligation to form parts to send to iGEM- Our aim was to add the following parts into Cm backbones to ship: Our four constructs that were in the fermenter ie. Alpha strong (min strong new); alpha weak (min weak new); beta strong (max strong one orginial) and beta weak (max weak two original). CPR and ADS (that had been synthesised by IDT) along with the repeat of putting ADH1 (no HisTags) into Biobrick form.

- Transformed the BioBrick ligations into DH5 alpha cells and also to transform the previously miniprepped pTAC into XL1 blue. pTAC red and white were being transformed into XL1 blue as XL1 blue are LAC inhibitor positive and we hoped this would help us to figure out if the pTAC from the kit had an RFP gene or not; and to decide which colour colony was the one we wanted and which was presumably an infection. We hoped to later ligate the correct pTAC to DBR2 to make our construct become IPTG inducible like the rest of the mevalonate pathway in B569 cells.

27th:

- Result of Transformations: All plates contained white colonies except the ADH1 plate which had red colonies exclusively. The mini prepped construct plates displayed more white colonies than those taken directly from the IDT synthesised DNA. We made broths of the white colonies.

28th:

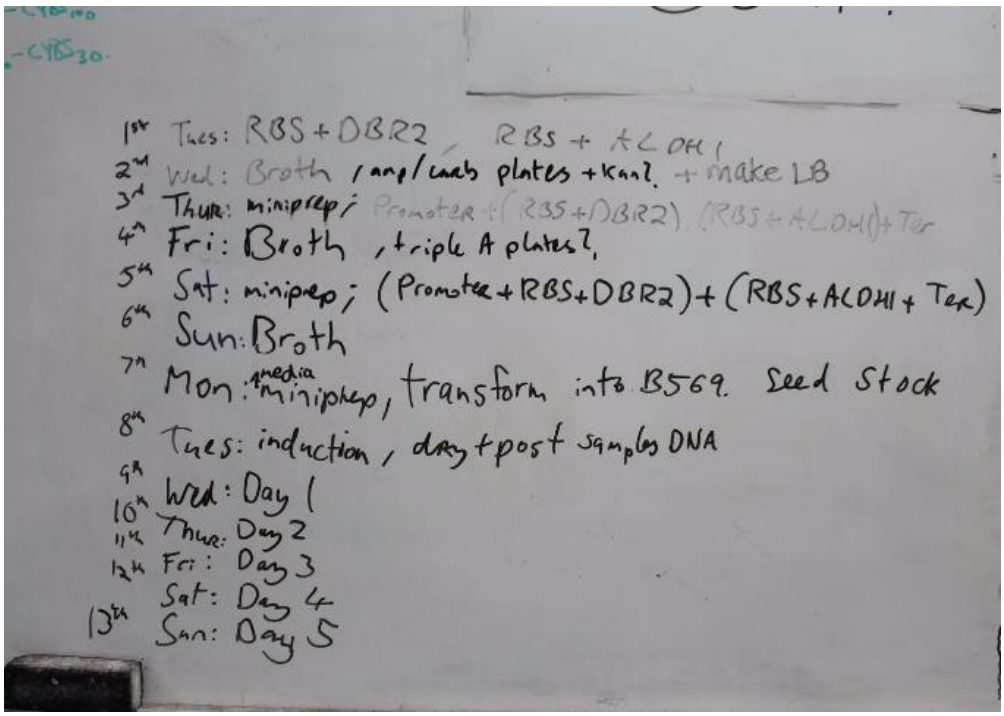
- Took samples of B569 (np) and Alpha Strong (Min Strong new) in preparation for GCMS.

- Repeat of putting ADH1 without His Tags into BioBrick form to ship ie. Into Cm backbone Planned to carry out duplicate using PCR product ADH1 and ADH1 direct from the IDT tube but there was found to be very little left in the IDT tube and so it was decided to carry out a duplicate with just the PCR product ADH1.

SEPTEMBER

2nd:

- Got sequencing results back. Unfortunately, none of the constructs had DBR2 integrated properly in them which turned out to be a major setback. Sad day for TCD iGEM
- Our old sequencing results showed even though DBR2 had been ligated successfully to RBS, ALDH1 wasn't ligating properly.
- We came up with a plan of action to complete a device composed of the following parts: Promoter + RBS strong + DBR2 + RBS weak + ALDH1 + Terminator; And to transform the construct into B569 to grow in the fermenter for 5 days. We were undeterred!

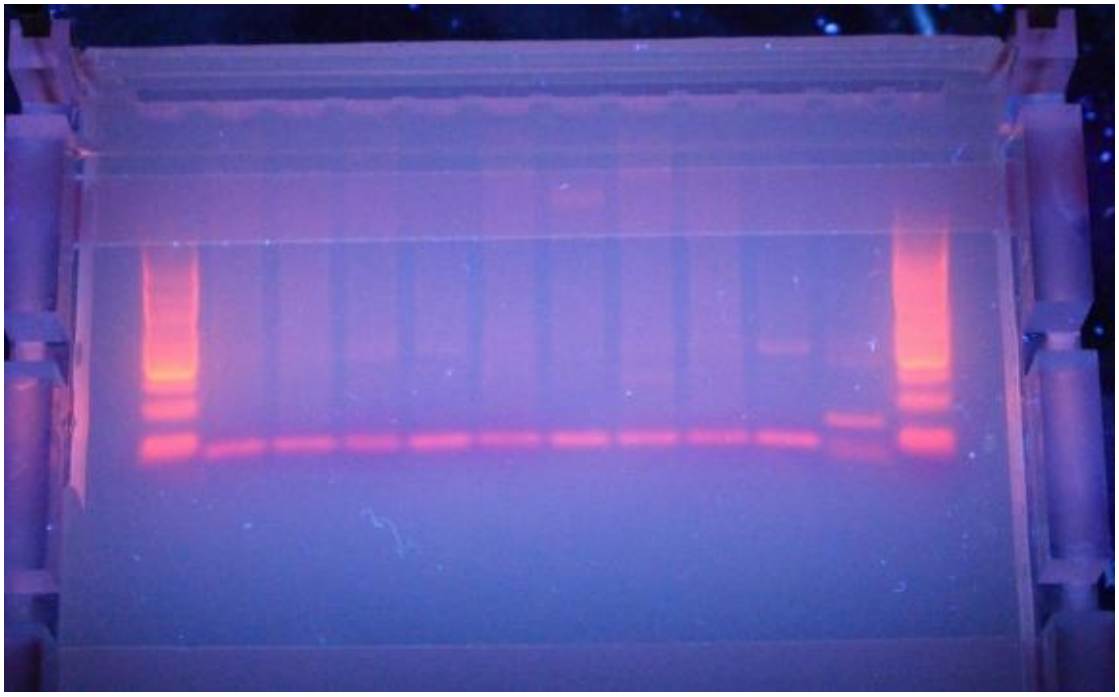


- We spoke to our supervisor Matthew Carrigan to get advice on how to save time. He recommended designing primers that could confirm whether or not a ligation had taken place and then running the PCR product on a gel. In retrospect we probably should have carried this out previously after every ligation so we wouldn't have been faced with the prospect of starting almost from scratch again.

- Started by the digestion, ligation and transformation of weak RBS (i.e. BBa_B0032) with ALDH1 and a Kanamycin backbone.

3rd:

- Carried out miniprep of the Weak RBS+ALDH1
- Carried out a PCR to determine if purified plasmid DNA contained the desired part. i.e. Weak RBS

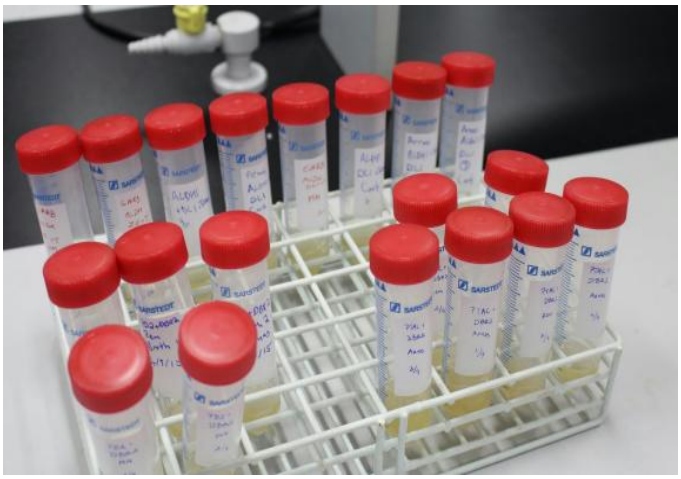


- Ran the PCR product on a gel to determine if weak Ribosome Binding Site (RBS) had successfully ligated to the AlDH1 Gene.
- Nine of the ten samples showed bands of the expected length. As the forward primer was designed to anneal to the weak ribosome binding site, the evidence from the gel suggested that in nine of the ten samples the PCR had been carried out successfully; This infers that in nine of the ten samples the previous ligation of the weak RBS to ALDH1 had been successful!!
- Carried out 3A assembly to add a terminator (BBa_B0015) to RBS + ALDH1 samples and to add promoters D2 (from Device 2 in the interlab study ie. BBa_K823008) and PTAC (BBa_K864400) to RBS (ie. BBa_B0034) + DBR2 A1. This was done in triplicate.

4th:

- The majority of the day was spent planning for the following day. Matt showed us how to operate the thermocycler and to use a 96 wellplate.

- Broths were prepared from all of the transformation plates made the previous day.



5th:

- The broths from the previous day were removed from the incubator in the morning
- Minprepped the samples and prepared two gels for PCR to confirm part size.
- PCR was done to determine if purified plasmid DNA contains the desired part. (.ie if RBS + ALDH1 is ligated to a terminator; if Promoter D2 (BBa_K823008) is ligated to RBS + DBR2; if PTAC promoter (BBa_K864400) is ligated to RBS + DBR2.
- Results of the gel:

Figure 1: 2% agarose gel: ran PCR products deriving from the primers A W_ALDH1 Term LP and AW_ALDH1 Term RP; PTAC+DBR2 LP and PTAC+DBR2 RP; A_p23106_S+DBR2 LP and A_p23106_S+DBR2 RP.

- It had been planned to ligate promoter+RBS+DBR2 to RBS+ALDH1+Terminator but based on the results of the gels it was decided not to proceed as from the evidence it appeared that none of the promoters had successfully ligated to RBS+DBR2.
- We discussed our options. Based on the last set of sequencing results it seemed that the step of ligating a promoter to RBS+DBR2 had also been a problem. We decided that we would attempt to ligate all the promoters that we had prepared to RBS+DBR2 and in parallel to ligate all of the promoters to the RBS+ALDH1+Terminator 1// sample that we thought had been successfully assembled.

6th:

- Ligated the promoters; D1 (Device 1 promoter interlab study K823005), D2 (K823008), D3 (K823013), PTAC (K864400) and PLAC (K864400) to RBS+DBR2 and RBS+ALDH1+Terminator.

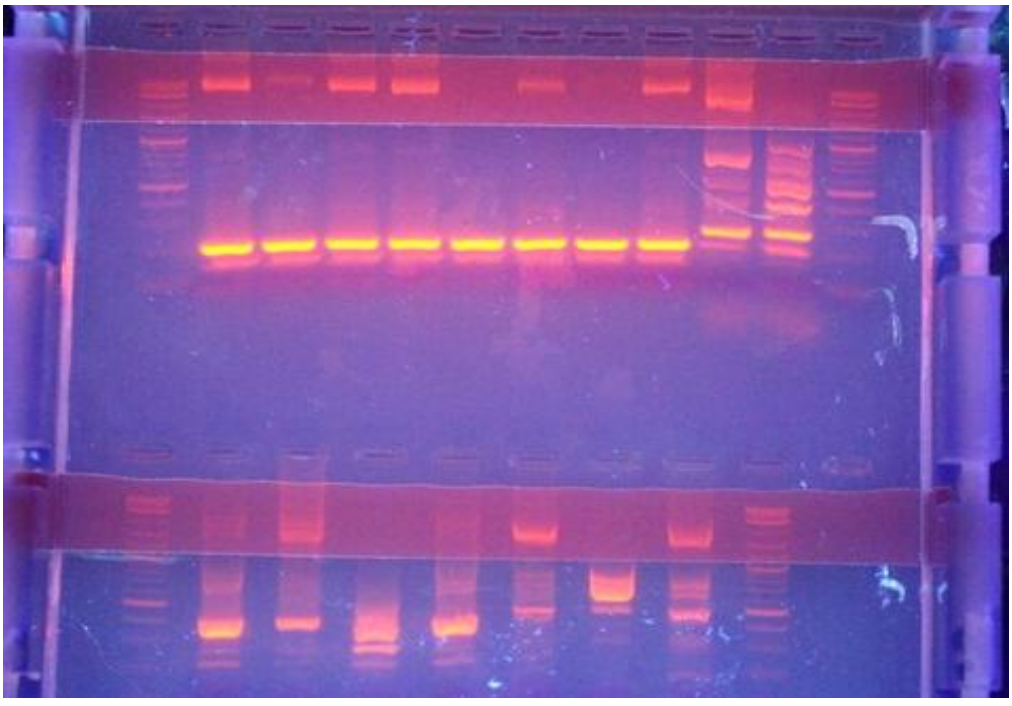
- Transformed the ligated products into DH5alpha cells so that purified DNA could be obtained via plating, broth and miniprep.

- Also to transform the hypothetical functioning device of promoter+RBS+ALDH1+Terminator directly into B569 cells, B569 cells that were previously obtained from Amyris (who engineered the cells to produce artemisinic acid, a precursor to artemisinin.) were used for transformation of the device.

- The cells were grown into a fermenter for a number of days and GCMS analysis to check levels of artemisinic acid versus a control of B569 cells without the device was carried out.

8^{th}:

- To determine if purified plasmid DNA contained the desired part. (i.e. if RBS + ALDH1+Terminator was ligated to any of the promoters D1, D2, D3, PTAC, PLAC in DH5alpha and also in B569 and; if any of the successfully miniprepped RBS + DBR2 samples had promoter D1 or D2, a PCR was carried out.
- Transformation of Promoter + RBS + ALDH1 + Terminator purified samples miniprepped from DH5alpha into B569 competent cells
- Transformed B569 cells with purified Promoter + RBS + ALDH1 + Terminator plasmid DNA from DH5alpha cells.
- Gel results of the B569 samples showed clear bands of the expected length and so were chosen to be inoculated directly into cultures to run in the fermenter.



- There were clear bands of expected length in the gel of (bottom row DH5alpha) Lane 8: PD1+RBS+ALDH1+Terminator Arnas and Lane 14: PTAC+RBS+ALDH1+Terminator Arnas. Based on this, 800µL of the transformation broth of these plasmids into B569 were inoculated into cultures to be run in the fermenter.
- The bottom row lane 3 (PDI+RBS+DBR2 Dáire 1), 4 (PD1+RBS+DBR2 Arnas) and 6 (PD2+RBS+DBR2 Dáire) showed clear bands of expected length. It was decided to use these samples for ligation to RBS+ALDH1+Terminator.

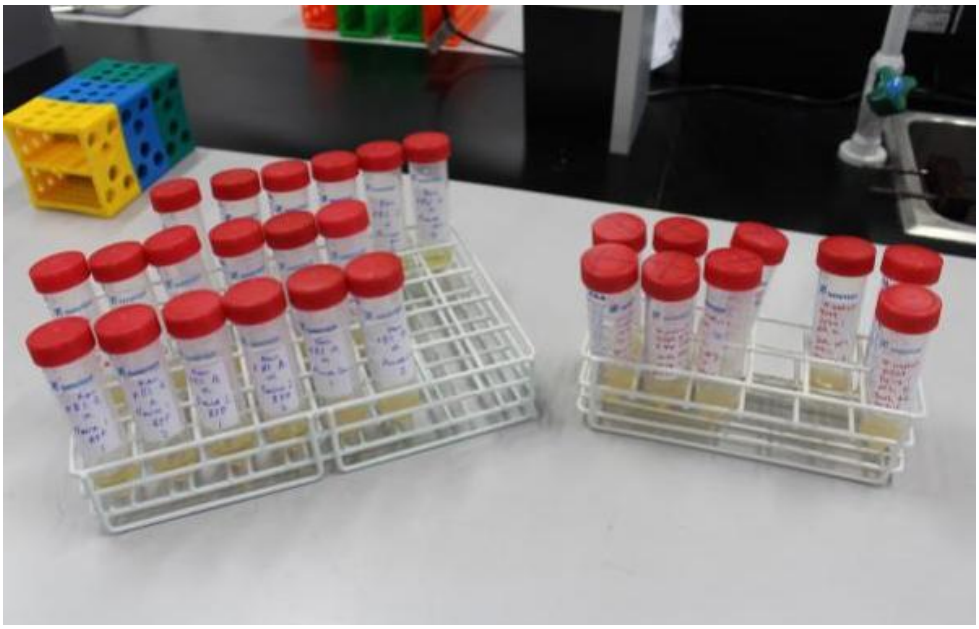
9th:

- Sent samples for sequencing

- * PD2 + RBS + ALDH1 + T D (Broth had been inoculated into culture)
- * PD3 + RBS + ALDH1 + T D (Broth had been inoculated into culture)
- * PD1 + RBS + ALDH1 + T Arnas (miniprep from DH5alpha was transformed into B569 and transformation SOB was inoculated directly into culture)
- * PTAC + RBS + ALDH1 + T Arnas (miniprep from DH5alpha was transformed into B569 and transformation SOB was inoculated directly into culture)
- Ligated and transformed Promoter+RBS+DBR2 samples to RBS+ALDH1+Terminator samples into DH5 alpha and B569 cells.

10th:

- Sequencing Results: PDI + RBS + DBR2 Arnas: Pairwise alignment (using Geneoius): 100% DBR2 and promoter; PDI + RBS + DBR2 Dáire 1: 100 % DBR2 and promoter; PD1 + RBS + ALDH1 + Terminator Arnas: 100% identity for promoter and terminator
- Preparation of Broths from all of the transformation plates made the previous day. (i.e. Promoter + RBS + DBR2 + RBS + ALDH1 + Terminator)



11th:

- Polymerase Chain Reaction (PCR) carried out to determine if purified plasmid DNA contained the desired device. ie. Promoter + RBS + DBR2 + RBS + ALDH1 + Terminator.
- Took a sample for GCMS analysis from the fermenter (Repeated daily after IPTG induction) to take a sample of the culture in the fermenter to later test for the presence of artemisinic acid.

- Ran PCR products on a 1% agarose gel to investigate if PCR products were the desired size, and to determine whether or not previous ligation of Promoter+RBS+DBR2 and RBS+ALDH1+Terminator had been successful.

- Based on the results it was decided to remove the current alpha constructs from the incubator and to transform PD1 D alpha D2 RFP 1; PD1 D alpha D1 RFP 1 and PD1 D alpha D1 RFP 2 miniprepped from DH5alpha cells into B569 cells.

12th:

- Digestion and Ligation to put intermediate parts and final constructs into chloramphenicol backbones for submission to iGEM HQ.

13th:

- Preparation of Broths of parts in chloramphenicol backbones to purify and submit to iGEM HQ.

14th:

- Carried out the debugging of the Basehunter system sent by Cork iGEM as part of our collaboration.

15th:

- Found many colonies of GFP and RFP in respective plates of the Basehunter system. Sent the data over to iGEM Cork who confirmed our results were as they expected.

16th:

- Documented the parts we had constructed and uploaded them on the iGEM HQ page.
- Created a draft version our introductory video for the wiki.

17th

- Took pictures of each member of the team
- Carried out GC/MS to validate the parts

18th

- GC/MS turned out to be successful!
- As the day of wiki freeze, we were finishing literally everything to put on our website.
- Got complimented by our supervisors!